A PRELIMINARY ACCOUNT OF THE CLEAVAGE OF ARENICOLA CRISTATA, WITH REMARKS ON THE MOSAIC THEORY.

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I. Cleavage.

The eggs in the jelly in which they were laid were fixed in picro-acetic acid and preserved in alcohol. This treatment renders the jelly perfectly soluble in distilled water, so that the eggs can easily be freed from the jelly by allowing a mass of it containing eggs to stand a few minutes in distilled water. The jelly disappears, and the eggs sink to the bottom. They are stained in dilute Delafield's haematoxylin, cleared and examined in clove-oil.

The individual egg is fairly well filled with small yolk spheres, which are scattered throughout all parts of it and are found in all the cells of the earlier stages. The cleavage conforms closely to the so-called "spiral" or "oblique" type, especially in its earlier stages. Later, as in the other forms of this type, cleavages which follow entirely different laws occur.

Figs. 1, 2, and 3 represent respectively the 2-, 4-, and 8-cell stages from the upper pole. In Fig. 1 both cells are already in division, and the spindles do not lie in the same plane, but are inclined to each other, so that this division is a true oblique segmentation. In Fig. 2 the second division has occurred, producing one enormous cell (*D*) and three much smaller ones (*A*, *B*, *C*). The large cell *D* is in contact with its opposite *B*, at both poles of the egg, *i.e.*, the two cross-furrows are parallel. The cross-furrow at the lower pole is longer than the other and perfectly constant up to a late stage, so that it furnishes an invaluable means of orientation.

It lies at right angles to the future median plane of the adult. The cell *D* is dorsal, *B* is ventral, and *A* and *C* are left

and right. The upper pole represents approximately the anterior end, and the lower pole the posterior end. In other words, the median plane of the adult passes through the blastomere *D* and forms an angle of 45° with the first two cleavage-planes. This orientation does not agree with that given by

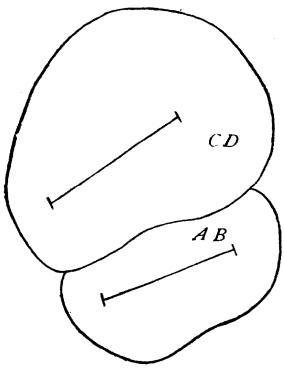

FIG. 1.

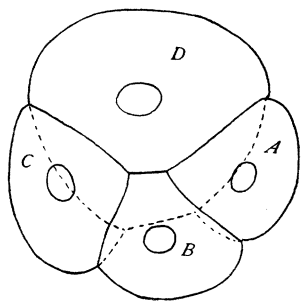

FIG. 2.

Wilson ('92) for *Nereis*, but I am unable to make his figures agree with his orientation. Mead ('94) gives the same orientation for *Amphitrite* that I find in *Arenicola*.

The enormous size of the blastomere *D* is interesting, as it of necessity influences the whole of the cleavage. This is the cell from which the first and second somatoblasts (*X* and *M*) will arise.

In Fig. 3, the 8-cell stage, it is seen that the four so-called micromeres are very large, the two dorsal ones being slightly larger than the ventral pair. The position of the posterior cross-furrow at this stage is considerably ventral to the posterior pole.

The cleavage proceeds in the typical oblique manner up to a stage of fifty-eight cells. During this period, the germ-layers have been separated. The ectoblasts arise as three "quartets" of blastomeres, given off successively and with alternating direction of spindle from the macromeres, the mesoblast (*M*) appears as a single cell, and the entomeres are represented by four cells. The large ectomere *X*, which is to furnish almost the whole ectoderm of the trunk, is seen in Fig. 4. The cells

of the first quartet of ectomeres have divided several times, and sixteen primary trochoblasts have been formed (Fig. 5, *tr* I; Fig. 6, *tr* I). Various divisions have occurred in other blastomeres except the mesoblast (Fig. 5, *M*). Figure 6 shows the passage from the 58-cell stage to the next from the upper pole. The four spindles here represent the first bilaterally symmetrical division and also the formation of the apical cross of eight cells. The second bilaterally symmetrical division in the egg occurs in the largest derivative of *X* at the 70-cell stage. It is followed immediately by the symmetrical division of *M* into right and left halves. At a stage when the egg consists of over a hundred cells, a bilaterally symmetrical division occurs in the entomeres, the last division that they undergo before the blastopore closes. It is an interesting fact that in each of the cases mentioned, *viz.*, the cells of the first quartet, the derivative of *X*, the mesoblast and the entomeres, this first bilaterally symmetrical division occurs in *cells of the same generation*, the eighth, counting the unsegmented egg as the first. The later derivatives of the cells just mentioned all divide symmetrically, though in derivatives of the first quartet, I have often seen what is apparently a partial return to the oblique type in the direction of the spindle. Other cells of the egg

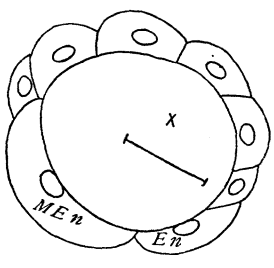

FIG. 3.

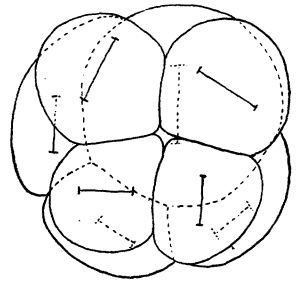

FIG. 4.

continue to divide obliquely up to a stage shortly before the closure of the blastopore, when, I think, all blastomeres are dividing symmetrically or approximately so. The sixteen primary trochoblasts do not divide further, but do not become ciliated until some time after the blastopore closes.

Fig. 7 shows a stage of considerably over 100 cells from the upper pole. The two cells which are lettered *N* are interesting because at this time they become very large, but lie rather deeply, so that they are partially covered by the rosette cells. Each then divides equally, the spindle forming an angle of 45° with the median plane, with its dorsal end nearest the median plane. After a period of rest both pairs divide again, but this time the more nearly median of each pair divides with a vertical spindle, *i.e.*, the ectoderm becomes two-layered here. Although I have been unable to follow these cells farther, it seems probable from their position and size that they may

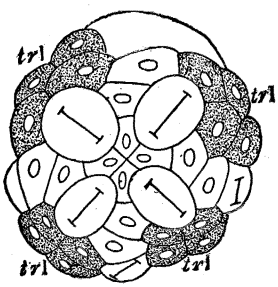

FIG. 5.

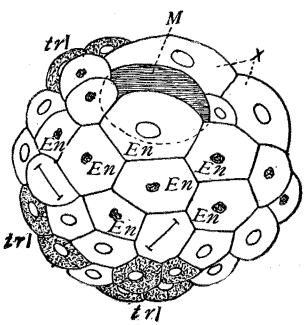

FIG. 6.

form the apical plate and thus the supraoesophageal ganglion or part of it. I do not believe that in *Arenicola* the rosette cells form the ganglion, though Wilson regards it as probable for *Nereis*.

The small cells lettered *K* correspond to the head-kidney cells of *Nereis;* I have been unable to discover a head kidney in *Arenicola*, and these cells remain as small, inconspicuous ectoderm cells.

The gastrulation in *Arenicola* is a combination of invagination and epiboly. The mesoblast begins the process by slowly elongating inward and gradually passing into the segmentation cavity which it just fills. Its bilateral division occurs during its passage. In Fig. 5 it is commencing already to pass inward. The entomeres are not enclosed until some hours later, although their superficial area is constantly decreasing. Opti-

cal sections show that they too are slowly elongating inward, and that their nuclei are sinking farther and farther from the surface of the egg. Finally, these cells extend as a column through the egg to the lower surface of the ectoderm of the upper pole (Fig. 9, *En*). As is seen by this figure, the amount of true overgrowth by the ectoderm is really slight, only the latest stages of the closure of the blastopore being effected in this way. The blastopore is at first nearly circular, but during the later stages of gastrulation it becomes dorso-ventrally elongated. To render this change in shape clear, the growth of the somatic plate must be explained.

The cells of the somatic plate, the derivatives of *X*, are at

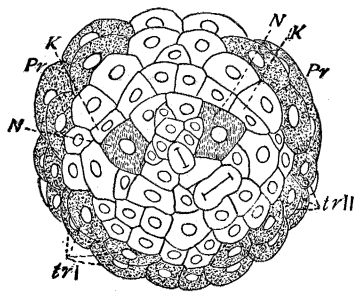

FIG. 7.

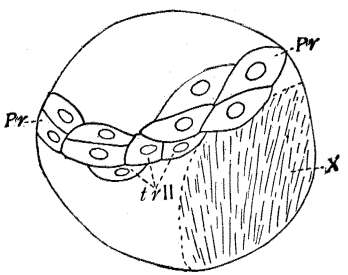

FIG. 8.

this time increasing rapidly in number, and the plate is extending itself posteriorly and laterally, the lateral growth being much more rapid than the posterior. It is this growth of the somatic plate that forces the sides of the blastopore together and finally causes them to meet. Fig. 10 shows the relations of blastopore and somatic plate at a stage shortly before closure. The lip of the blastopore, except the very narrow space dorsally where it is formed by the derivatives of *X*, consists of twelve cells (*St*), four along each side and four ventrally. The cells are all derivatives of the third quartet of ectomeres. I have followed their lineage exactly. At first they alternate with cells of the second quartet, but by a series of divisions they finally pass below these and then, dividing laterally, shut them off entirely from the actual lip of the blastopore. All the other cells shown in Fig. 10 are derivatives of *X*.

The closure of the blastopore begins at the dorsal end. The growth of the somatic plate (*X*) results finally in a concrescence of its two sides along the median line. The first two cells to meet are derivatives of those called *Xr*, *Xl*, in Fig. 10. Just dorsal to their point of meeting lies the proctodaeal region (*Proct* Fig. 10). This, though now filled with small cells, I believe must be regarded as in reality a part of the blastopore. The ventral portion of the blastopore, the portion still open in Fig. 10, later forms the stomodaeum. The distance between the two is continually increased by the ventrally advancing concrescence of the somatic plate. The cells of the

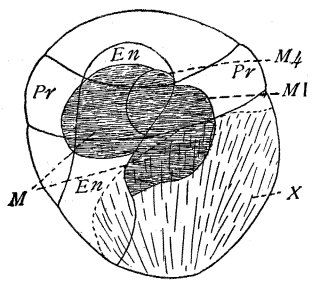

FIG. 9.

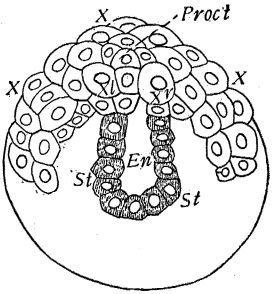

FIG. 10.

blastopore lip are pushed together from behind and from each side, principally the latter, until closure is effected. The somatic plate continues to grow posteriorly, carrying at its tip the proctodaeal region and the cells surrounding it, and thus the line of concrescence between the stomodaeum and proctodaeum increases in length, and growth in length of the larva begins. The area covered by the somatic plate in different stages is shown from the side in Figs. 8, 9, and 12, *X*.

The center of the blastopore from the earliest stages is ventral to the posterior pole, consequently the gastrula is not radially but bilaterally symmetrical, and its main axis does not correspond exactly with any of the future axes. These facts are easily deduced from Figs. 9 and 11.

About as soon as the two mesoblasts are completely inclosed, they begin the formation of the mesoblast bands. The spindle for the first division lies almost dorso-ventrally, the smaller

cell being given off ventrally and somewhat laterally. In each succeeding division, however, the direction of the spindle changes, the daughter cell arising each time more toward the upper pole, until finally at the stage of the blastopore closure the spindles of the mesoblasts are nearly longitudinal. Thus the main axis of the mesoblast band has changed by nearly 90°, but cells given off do not change their own position. Consequently, the anterior ends of the bands are curved ventrally. The position of the right mesoblast and its latest product are shown in Fig. 9, *M* 1 and *M* 4, while the rest of the shaded area shows approximately the extent of that portion of the right mesoblast band already formed. At this stage the change in direction is about half completed. Fig. 11 shows the relations of the layers in optical section just before closure of the blastopore, as seen from the ventral side and slightly anteriorly. The ends of the mesoblast bands (*M*) are seen on each side of the endodermic column.

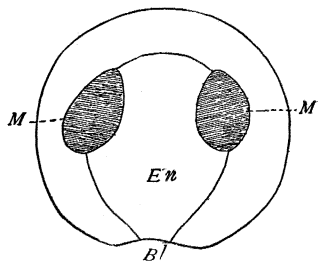

FIG. 11.

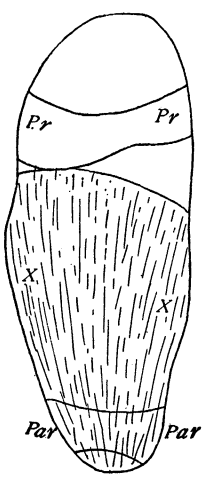

FIG. 12.

As stated above, the sixteen primary trochoblasts (Fig. 6, *tr* I) constitute the first indication of the prototroch. Later these are supplemented by nine cells from the second quartet (Fig. 7, *tr* II), three from each quadrant, except the dorsal, where a gap remains until about the time of the blastopore-closure. Fig. 8 shows the cells of the prototroch of the right side, the three secondary trochoblasts being lettered *tr* II. All the trochal cells elongate greatly, and finally the dorsal gap is closed, but before its closure four cells arising from the first quartet of ectomeres pass through it and are shut off by its closure from all connection with the pretrochal region.

The larva of *Arenicola* possesses a paratroch (Fig. 12, *Par*), but it is not functional until a very late stage.

Dr. Mead has very kindly furnished me with the lineage of the paratroch of *Amphitrite*, and it is interesting to note that the origin of this organ in the two cases is entirely different. The paratroch of *Arenicola* arises from certain derivatives of *X*, but not only is the series of divisions different in direction from that found in *Amphitrite*, but the cells, or a part of them, pass through several more generations than in *Amphitrite*.

The free-swimming trochopore of *Arenicola* already possesses three trunk segments with setae. The pelagic life does not last over two days. The larva[1] sinks to the bottom and crawls slowly about, and the growth of new segments begins. Fig. 12 is an outline of the trochopore in lateral view, the segmentation not being shown. The object of the figure is to show the area of the ectoderm formed by *X*.

Just a word here in regard to the cleavage of *Sternaspis scutata*. My work on this worm was begun at Naples, but, owing to loss of material, I have never yet been able to complete it. I desire to express my sincerest thanks to Professor Agassiz for the privilege of the Agassiz table at Naples, and also to Professors Dohrn and Eisig, as well as the other members of the staff of the Zoölogical Station, for their kindness during my stay. The unsegmented egg of *Sternaspis* is almost completely filled with very large yolk-spheres, but none of them pass into the ectomeres or the mesoblast. All these cells are relatively small, while the entomeres remain as enormous, yolk-packed cells. Yet up to a stage of about eighty cells (as far as I have been able to follow the cleavage of *Sternaspis*) the succession of cleavages is cell for cell the same as that of *Arenicola*. Sixteen cells are formed, corresponding to the primary trochoblasts of *Arenicola*, but *Sternaspis* has no prototroch, and they simply form a part of the ectoderm. On the other hand, the larva never really resembles a trochopore. It is not free-swimming. Vejdovsky ('81) states that the larvae at Trieste

[1] The larvae are very easily kept alive in covered glass dishes with a supply of *ulva*. I have kept them in the laboratory for three months, and thus have been enabled to preserve a complete series of stages of the late larval period and the metamorphosis.

were ciliated, but I have never been able to observe any cilia or any swimming motion in those raised at Naples. The larva simply elongates and bursts the egg membrane and then twists and squirms along the bottom and soon bores into the mud. Vejdovsky's account of the cleavage is also extremely superficial and careless.

To sum up: as regards orientation, *Arenicola* and *Sternaspis* both agree with *Amphitrite*, but not with *Nereis*. As regards the formation of the prototroch and the concrescence of the somatic plate, *Arenicola* and *Amphitrite* agree, but both differ from *Nereis*. *Arenicola* differs from *Amphitrite* in the origin of the paratroch and in various details, and from *Nereis* in the origin of the lips of the blastopore.

The preceding account has dealt only with the cytogenetic side of the question. The study of the cleavage has brought out a number of facts of cytological interest as well. Suffice it for the present, however, to say that cleavages occur which appear to contradict all the so-called laws for direction of spindle, etc.

II. The Mosaic Theory.

Wilson ('93a) regards the cleavage of *Nereis* as "a visible mosaic-work," and further asserts that "the principle of organ-forming germ regions has here a real meaning and value, and this would remain true even if hereafter it should be shown that both of the first two blastomeres of *Nereis*, if isolated, could produce a perfect embryo."

However, if the production of a whole embryo from a half-egg be possible, then the fate of the blastomeres is really after all "a function of their position." Wilson ('93a) suggested the possibility or probability of cellular interaction, thus departing from the true mosaic theory of Roux, of which the fundamental principle is self-differentiation. He still postulated, however, true morphogenetic differentiation in the blastomeres. More lately, as a result of Crampton's ('96a) experimental work on *Illyonassa*, he ('96b) has reaffirmed his views.

Lillie ('95) went so far as to say "the more precocious the differentiation of the organs of the somatoblast, the greater the

difference in the size of the cells (*i.e.*, the first two cells of cleavage). The two cells may be equal in size when the organs in question are not precociously developed. The same principles suffice to explain unequal divisions throughout the cytogeny."

My work on *Arenicola* and *Sternaspis*, together with a comparison of previous work, has led me to somewhat different conclusions. The oblique cleavage does not appear to be so strictly or so simply a mosaic as has been supposed.

First, corresponding cells differ greatly in size and structure in different forms without any corresponding differences in time of differentiation. To mention a few examples: In *Arenicola*, where the unsegmented egg contains much yolk, equally distributed, all the cells of several generations, including ectomeres, first somatoblast, and mesoblast, acquire yolk granules. The ectomeres are very large, and the first somatoblast and mesoblast are the largest cells in the egg, leaving the entomeres quite small. In *Sternaspis*, where the egg is even more closely packed with yolk, it is all retained in the entomeres, and these are enormous, leaving the ectomeres and the mesoblast as small, protoplasmic cells.

Yet in both these cases the cleavage is cell for cell the same up to a late stage, and the differences in the order of cleavage are not great. In general, the cells have the same fate, except that *Sternaspis* has no prototroch.

Comparisons along this line may easily be carried further, but this is perhaps sufficient to show that the appearance of a large blastomere does not necessarily imply that it is packed with precociously differentiated material.

Secondly, as the number of studies of the oblique cleavage increases, it becomes more and more evident that cell homology is not a true homology. *Sternaspis* never possesses a prototroch, but large cells corresponding to the primary trochoblasts of *Arenicola* are formed, and every division up to the differentiation of the trochoblasts in *Arenicola* is exactly paralleled by *Sternaspis*. Again, *Arenicola* and *Amphitrite* both possess a paratroch, but in *Arenicola* the cells forming it arise by a different series of divisions and pass through several

more generations than do the paratrochal cells in *Amphitrite*. A cell in the segmenting egg of *Arenicola* arises in exactly the same manner as the head-kidney cell of *Nereis*, but remains as a part of the ectoderm.

These facts seem to bear more or less directly on the question of the mosaic theory, for, if we have a true mosaic in these cases with so great uniformity of cleavage up to a stage of a hundred cells or so, may we not expect to find a somewhat closely corresponding uniformity in the fate of the blastomeres? Of course the view is possible that each egg is, so to speak, laid out in a different mosaic, even though the forms of cleavage may correspond, but is it probable?

There is, however, a third argument against the mosaic theory as related to the oblique cleavage, which seems more conclusive than either of the preceding.

About a year ago Crampton's ('96a) experimental work on *Illyonassa* appeared. It was followed by an appendix by Wilson ('96b). In the latter paper Wilson asserts that the results of Crampton's work confirm his view that the cleavage of *Nereis* is "a visible mosaic-work." Crampton succeeded in separating the segmenting egg of *Illyonassa* into halves, quarters, and eighths, which were capable of further development. He asserts that the parts, with the exception of a few details, segment exactly as they would if the rest of the egg were present, and that therefore *Illyonassa* possesses no "postgenerative" or "regenerative" power, and that the cleavage is a mosaic. He noticed, it is true, a few changes in direction of cleavage, or in size of the products in the partial embryos, but explains them as the result of lack of pressure, owing to the absence of the other blastomeres. If differences of pressure are able to change the form of cleavage so easily here, it is strange that changes do not occur in the normal cleavage where in many cases, as far as can be seen, the spindles lie nearly or quite in the direction of greatest pressure, and the division pushes whole groups of cells from their positions temporarily. Furthermore, in no case was Crampton able to bring the larvae up to a stage late enough to determine that "regeneration" could not take place. He did, it is true, obtain partial

circles of cilia instead of complete ones, but in only one case, and then indefinitely, does he give the number of ciliated cells. In no case were mesoblast-bands formed, even where they might be expected. This early death shows at any rate the extreme dependence of one portion of the embryo upon another.

Finally, and this is the crucial point, from a study of his paper it appears that "regeneration" has in most cases actually occurred. In no single instance does the partial larva retain the form which the part would have in the whole, though doubtless this is due in part to the rounding out of cells in consequence of the absence of external pressure. But the ectomeres always become more or less changed. They form a rounded cap, not a half or a quarter of such a cap. Lastly the ectomeres or cells which, in the normal cleavage, come to cover the *outer side* of half the entomeres, in the half embryo, *completely overgrow these entomeres, thus forming a gastrula and bringing the blastopore to closure.* In his description of the half embryos Crampton says: "Ectomeric divisions continue, the ectodermic cap grows lower, as shown in Fig. 18, before another division of *A* and *B* takes place. After the fourth division of these viewed from the lower pole there are four cells easily recognizable as entodermic. These are finally completely overgrown by the ectodermic cells, and in one case, sixteen hours after isolation, a partial circle of cilia was developed." Again, in speaking of the ¼ blastomeres, he says: "Throughout later development, two large cells containing yolk matter are plainly seen, inclosed by the clear ectodermic cells." Still again, in another embryo of the same sort: "The two entomeres are still distinctly visible, surrounded by ectoderm cells." In his figures the same point is shown several times.

Now, in order to accomplish this complete overgrowth and inclosure of the entomeres, either the ectodermal cells have changed their method of cleavage and come to lie where normally they would not, or else more cells than the normal number have arisen from the macromeres.

Unfortunately, only the most general statements are given regarding the later development, but in any case the larvae are

not ½ or ¼ larvae, but have become more or less complete wholes. Wilson appears to have overlooked this point entirely, in quoting this work in support of his views. He asserts that "apart from a few unimportant details, blastomeres of the two-cell or four-cell stage, whether isolated singly or in groups, segment precisely as if the missing portions of the egg were present, and the resulting larval fragment is completely devoid, not only of the power of a regulatory rearrangement of its material, but also of regenerative or post-generative power. The cleavage is thus demonstrated to be in the gasteropod precisely what I have asserted that of *Nereis* to be, *viz.*, "a visible mosaic-work."

Even here, then, in this highly specialized type of cleavage, the fate of the cells has been altered by a change of conditions. If we accept Roux's "Reserveidioplasson," it is of course possible to escape from this difficulty.

The oblique cleavage is not, then, a direct division of the egg into so many sets of organs which exist as "Anlagen" in the cells, but the organs are differentiated as the result of processes going on in the egg as a whole, though, later in development, self-differentiation may occur to some extent. The cell is after all no true morphological unit in cleavage, as, indeed, an increasing number of facts is showing us, *e.g.*, the papers of Hammar ('97b) and Andrews ('97a), and it is difficult to believe in the face of these and other facts that it is physiologically isolated.

I believe that any theory of development, proceeding on a strictly cellular basis, must fail in its attempt to explain ontogeny. The organism from the unsegmented egg to the adult is a whole, and at every stage of its existence acts as such ('93b). There is often visible organization in the unsegmented egg, but this, by no means, corresponds to the adult organization. That organization is the final result of the processes constantly going on in the developing egg, and changes occurring in one part are the result of the preceding changes in the other parts and in their turn become causes of others.

Cleavage, however, if not necessarily cell-differentiation, is not merely a mechanical splitting up of the egg, nor is its form

determined, as Hertwig states ('97c) by the arrangement of the yolk. As was pointed out above, the yolk, though distributed throughout the unsegmented egg, may go to all the cells, and yet these cells may exhibit great differences in size, or it may appear in only a small number of them. Both the form of cleavage and the distribution of the egg-substance are determined by some power in the egg, which acts, at least in part, according to laws which are not yet understood. This power constitutes the true organization of the egg. The fertilized egg is not divided into pigeon-holes containing substances for different portions of the adult body, but it is simply a cell possessing the power to initiate certain chemical and physical processes, which in their turn initiate others, until, as the final result, the adult appears. This is epigenesis pure and simple, but it differs from Hertwig's position in that it recognizes a more fundamental organization in the egg than the visible one consisting of protoplasm and deutoplasm.

This organization is in general terms the specific nature of the reproductive cell. It is a phase of the same power that determines that the egg of *Arenicola cristata* shall develop into *Arenicola cristata*. At present we are in the dark concerning it. According to Weismann it is the germ-plasm, but whatever it be called, it is present from the beginning, and the visible cytoplasmic organization, the form of cleavage, and the whole ontogeny are the result of it.

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April, 1897.

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